

**B. In the Claims**

Please cancel claims 1 to 24 without prejudice. Please enter new claims 25 to 65.

Upon entry of the present amendment, the status of the claims will be as follows:

Claims 1-24 (Canceled).

25. (New) A method for detecting the presence of a target myostatin variant nucleic acid sequence in a nucleic acid-containing specimen wherein the specimen is from a non-human subject having increased muscle mass or having a predisposition for increased muscle mass as compared to a subject having a wild-type nucleic acid sequence, said method comprising detecting the presence of the target myostatin variant nucleotide sequence, wherein the presence of the variant target nucleotide sequence is indicative of a increased muscle mass or a predisposition for increased muscle mass.

26. (New) The method of claim 25, further comprising amplifying the nucleic acid prior to detecting.

27. (New) The method of claim 26, wherein the amplification is by means of oligonucleotides which hybridize to flanking regions of the target nucleic acid.

28. (New) The method of claim 25, wherein the variant target nucleic acid comprises a mutation, a restriction fragment length polymorphism, a nucleic acid deletion, a nucleic acid substitution or any combination thereof.

29. (New) The method of claim 28, wherein the nucleic acid deletion results in a truncated protein.

30. (New) The method of claim 28, wherein the mutation is a G to A substitution at nucleotide 1056 of a myostatin gene corresponding to nucleotide 1056 in GenBank Accession No. AFO19620, or a corresponding nucleotide in a myostatin gene.

31. (New) The method of claim 27, wherein the nucleotide sequence of the flanking regions to which the oligonucleotides hybridize is:

5'GATCCCAAACACTCTCCTACCTCGGATCCGCG-3' (SEQ ID NO:1); and

5'-CCCCTCAACAATTTTGAAACTGTGGGATCCGCG-3' (SEQ ID NO:2).

32. (New) The method of claim 31, wherein the oligonucleotides are:

5'-CGCGGATCCGAGGTAGGAGAGTGTTTTGGGATC-3' (SEQ ID NO:3);

and

5'-CGCGGATCCCACAGTTTCAAATTGTTGAGGGG-3' (SEQ ID NO:4).

33. (New) The method of claim 25, wherein the target nucleic acid is detected using a nucleic acid hybridization probe.

34. (New) The method of claim 33, wherein the target nucleic acid to which the nucleic acid hybridization probe hybridizes is selected from:  
5'-GTGGAGTGTTCAT-3' (SEQ ID NO:5); and  
5'-GATTCTGTCACAA-3' (SEQ ID NO:6); or  
5'-AATTCACATTCTC-3' (SEQ ID NO:7); and  
5'-AATTCATATTCTC-3' (SEQ ID NO:8).
35. (New) The method of claim 33, wherein the nucleic acid hybridization probe is selected from:  
5'-ATGAACACTCCAC-3' (SEQ ID NO:9); and  
5'-TTGTGACAGAATC-3' (SEQ ID NO:10); or  
5'-GAGAATGTGAATT-3' (SEQ ID NO:11); and  
5'-GAGAATATGAATT-3' (SEQ ID NO:12), respectively.
36. (New) The method of claim 25, wherein the subject is an avian, bovine, ovine, piscine, baboon, murine, or porcine.
37. (New) The method of claim 36, wherein the subject is of the species bovine.
38. (New) The method of claim 36, wherein the subject is of the species avian.
39. (New) The method of claim 38, wherein the avian is a chicken or a turkey.
40. (New) The method claim 25, wherein the specimen is a food product.

41. (New) The method of claim 25, wherein the subject is heterozygous for the variant myostatin nucleic acid sequence.

42. (New) The method of claim 25, wherein the subject is homozygous for the variant myostatin nucleic acid sequence.

43. (New) A kit useful for the detection of a target nucleic acid sequence in a specimen from a subject having increased muscle mass as compared to a subject having a wild-type nucleic acid sequence or having a predisposition for increased muscle mass, wherein the presence of the target nucleic acid sequence in the specimen is indicative of having or predisposed to having increased muscle mass, the kit comprising one or more containers comprising a first container containing a nucleic acid hybridization probe, wherein the probe hybridizes to a target nucleic acid selected from:

5'-GTGGAGTGTTTCAT-3' (SEQ ID NO:5); and

5'-GATTCTGTCACAA-3' (SEQ ID NO:6); or

5'-AATTCACATTCTC-3' (SEQ ID NO:7); and

5'-AATTCATATTCTC-3' (SEQ ID NO:8); and

a second container containing a means for detecting hybridization of the probe with the target nucleic acid.

44. (New) The kit of claim 43, wherein the nucleic acid hybridization probe is selected from:

5'-ATGAACACTCCAC-3' (SEQ ID NO:9); and  
5'-TTGTGACAGAATC-3' (SEQ ID NO:10); or  
5'-GAGAATGTGAATT-3' (SEQ ID NO:11); and  
5'-GAGAATATGAATT-3' (SEQ ID NO:12), respectively.

45. (New) The kit of claim 43, further comprising an amplification polymerase and deoxyribonucleotide(s).

46. (New) The kit of claim 43, wherein the detectable means is selected from the group consisting of enzymes, chemiluminescers, radionuclides, fluorescent compounds, heavy metals and ligands.

47. (New) The kit of claim 43, further comprising a third container containing oligonucleotides which hybridize to the flanking regions of a target nucleic acid, wherein the oligonucleotides hybridize to a nucleic acid having a sequence of:

5'-GATCCCAAACACTCTCCTACCTCGGATCCGCG-3' (SEQ ID NO:1);  
5'-CCCCTCAACAATTTTGAACTGTGGGATCCGCG-3' (SEQ ID NO:2).

48. (New) The kit of claim 43, further comprising a third container containing oligonucleotides which hybridize to the flanking regions of a target nucleic acid, wherein the oligonucleotides are:

5'-CGCGGATCCGAGGTAGGAGAGTGTTTTGGGATC-3' (SEQ ID NO: 3);  
and  
5'-CGCGGATCCCACAGTTTCAAAATTGTTGAGGGG-3' (SEQ ID NO: 4).

49. (New) A kit useful for the detection of a target nucleic acid sequence in a specimen from a subject having increased muscle mass as compared to a subject having a wild-type nucleic acid sequence or having a predisposition for increased muscle mass, wherein the presence of the target nucleic acid sequence in the specimen is indicative of having or predisposed to having increased muscle mass, the kit comprising carrier means being compartmentalized to receive in close confinement therein one or more containers comprising a first container containing oligonucleotides which hybridize to the flanking regions of a target nucleic acid, wherein the oligonucleotides hybridize to a nucleic acid having a sequence of:

5'-GATCCCCAAAACACTCTCCTACCTCGGATCCGCG-3' (SEQ ID NO:1);

5'-CCCCTCAACAATTTTGAAACTGTGGGATCCGCG-3' (SEQ ID NO:2).

50. (New) The kit of claim 45, wherein the oligonucleotides are:

5'-CGCGGATCCGAGGTAGGAGAGTGTTTTGGGATC-3' (SEQ ID NO:3);

and

5'-CGCGGATCCCACAGTTTCAAATTGTTGAGGGG-3' (SEQ ID NO:4).

51. (New) A kit useful for the detection of a variant myostatin polypeptide in a specimen from a subject having increased muscle mass as compared to a subject having a wild-type nucleic acid sequence or having a predisposition for increased muscle mass, the kit comprising one or more containers comprising a container containing an antibody which binds to amino acid residues 1-273 of wild-type myostatin polypeptide.

52. (New) A kit useful for the detection of a variant myostatin polypeptide in a specimen from a subject having increased muscle mass as compared to a subject having a wild-type nucleic acid sequence or having a predisposition for increased muscle mass, the kit comprising one or more containers comprising a container containing an antibody which binds to amino acid residues 274-375 of wild-type myostatin polypeptide.

53. (New) A kit useful for the detection of a target nucleic acid sequence in a specimen from a vertebrate having, or predisposed to having, the double muscling phenotype, wherein the presence of the target nucleic acid sequence in the specimen is indicative of having or predisposed to having the double muscling phenotype, the kit comprising carrier means being compartmentalized to receive in close confinement therein one or more containers comprising a first container containing oligonucleotides which hybridize to the flanking regions of a target nucleic acid, wherein the oligonucleotides are:

5'-CGCGGATCCGAGGTAGGAGAGTGTTTTGGGATC-3' (SEQ ID NO: 3);

and

5'-CGCGGATCCCACAGTTTCAAATTGTTGAGGGG-3' (SEQ ID NO: 4).

54. (New) The method of claim 37, wherein the subject is a Belgian Blue or Piedmontese breed.

55. (New) The method of claim 25, wherein the specimen is muscle tissue.

56. (New) The method of claim 55, wherein the tissue is skeletal muscle tissue.

57. (New) A method for identifying a nucleotide sequence of a naturally occurring mutant of a gene which normally encodes a myostatin protein, of a mammal displaying muscular hyperplasia, the method comprising:

obtaining a sample of material containing DNA from the mammal; and  
contacting the sample with a nucleic acid probe based on a nucleotide sequence of a known gene encoding myostatin in order to identify the nucleotide sequence of the mutant gene.

58. (New) The method of claim 57, wherein the probe binds to a nucleotide sequence identified as SEQ ID NO:5, 6, 7 or 8.

59. (New) The method of claim 58, wherein the probe is based on SEQ ID NO:11 or SEQ ID NO:12.

60. (New) The method of claim 59, wherein the probe is at least 10-50 nucleotides in length.

61. (New) The method of claim 57, wherein the step of probing the sample includes exposing the DNA to the probe under hybridizing conditions and further comprising isolating hybridized nucleic acid molecules.

62. (New) The method of claim 61, further comprising the step of sequencing isolated DNA.



In re Application of:  
Lee and McPherron  
Application No.: Unassigned  
Filed: September 15, 2003  
Page 11

PATENT  
Attorney Docket No.: JHU1410-1

63. (New) The method of claim 57, wherein the mammal is a bovine mammal and the probe is based on a said nucleotide sequence identified as GenBank Accession No. AFO19620.

64. (New) The method of claim 62, further comprising the step of isolating and sequencing a cDNA or mRNA encoding the complete mutant myostatin protein.

65. (New) The method of claim 64, further comprising the step of isolating and sequencing a functional wild type myostatin from a said mammal not displaying muscular hyperplasia.